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On the Decomposition of Lignin and Cellulose in Litter caused by Soil-inhabiting Hymenomycetes.

By

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Introduction.

With the residues of dead plants the soil is being annually supplied with considerable quantities of lignin. Among the major constituents of the cell walls the most resistant to biological decomposition is undoubtedly lignin. The decomposition of this substance, however, is of fundamental importance for continued production.

Our knowledge of the organisms that attack the lignin in the soil is still very incomplete. In forest soil, however, as FALCK (1923, 1930) was the first to point out, the Basidiomycetes probably play an important part in the breakdown of this substance. FALCK (1930) demonstrated experimentally that *Clitocybe nebularis* decomposes lignin as well as cellulose in litter, the lignin being broken down more intensely than the cellulose, so that a certain amount of cellulose is liberated (white rot). On the other hand, FALCK found that certain *Coniophora* species attack only the cellulose but leave the lignin (brown rot). He assumed that several soil Hymenomycetes behave like *Clitocybe nebularis*, others like the *Coniophora* species. WAKSMAN and MCGRATH (1931) and WAKSMAN (1931) showed that certain coprophile Hymenomycetes, *viz. Psalliota campestris* and *Coprinus radians*, decompose lignin as well as cellulose in dead plant material. The present writer, finally, was able to show that several *Marasmius* species occurring in nature on decaying plant residues decompose both lignin and cellulose (LINDEBERG 1944). This held true for 13 of the 14 species investigated. Certain species, *e. g. M. putillus*, *M. peronatus* and *M. androsaceus*, decomposed proportionally two to three times more lignin than cellulose, thus causing a considerable removal of incrusting lignin from the cellulose.

In the present work a report is submitted of some experimental investigations on the influence that a rather large number of soil Hymenomycetes exercise on lignin and cellulose in sterilized plant material. The objects in view have been: (1) to contribute to widening our knowledge of the occurrence among soil Hymenomycetes of species capable of decomposing lignin, (2) to identify such species as decompose the lignin to a great extent while breaking down the cellulose to a relatively small extent, *i. e.* species that cause a rather considerable liberation of the cellulose. Such extreme lignin decomposers might conceivably find technical use for the removal of lignin from cellulose by a biological method. They could no doubt also be of value as suitable test organisms in the investigation of enzymes active in the biological decomposition of lignin and the intermediary substances arising in this process.

The experiments have consisted in inoculating pure cultures of various fungi on definite quantities of sterilized plant material, whose lignin and cellulose contents have been determined at the beginning and end of the experiments.

Methods.

Gathering and cultivation of soil fungi. During the autumn of 1943 collections were made of a large number of soil Hymenomycetes which, on account of their mode of occurrence in nature, could be assumed to possess the capability of decomposing the more resistant constituents of dead plant material. Mainly such species as grow on decaying leaves were selected. Some fungi growing on stubs were also collected.

Pure cultures of the fungi were obtained by routine methods, either by inducing spores to germinate in malt extract agar containing 2.5 % malt extract and 1.5 % agar-agar or else by taking pieces of tissue from the fruit-bodies and growing them on sterile substrates (malt extract agar or nutrient solutions).

Cultivation of the fungi on sterilized litter. As substrates dead plant material (litter) was used of the following kinds:

1. Dead leaves of beech (*Fagus silvatica*) which had remained on the trees during the winter, gathered in April, 1942, direct from the trees in the beech wood at Maltesholm, Ö. Sönnarslöv, Skåne.

2. Straw and leaves of *Glyceria maxima* (preceding year's plants), gathered in April 1938 at Ultuna, Uppsala.

After the water content of the plant material in an air-dried state had been determined, fixed quantities of the material (see Tables 2 and 5) were introduced into 300 ml Erlenmeyer flasks. To each flask 50 ml of distilled water were added, whereupon the flasks were furnished with cotton stoppers and autoclaved (120° C.).

The plant material was inoculated with pure cultures of the fungi grown on malt extract agar. Four parallel flasks were inoculated with each species. The cultures were thereupon stored for a certain time (see Tables 2 and 5) at 25° C. Water losses from the flasks were controlled during the experimental period by weighing non-inoculated control flasks every other month, the loss being then replaced in all the flasks by addition of sterile water. Concomitantly with these additions of water the plant material in the flasks was loosened up by agitation with a sterile inoculating needle. At the end of the experimental period the contents of the flasks were transferred to weighed glass dishes and then dried at 60—65° C. for three days. Thereupon the dishes with their contents were allowed to stand in the laboratory another three days to enable the plant material to attain a humidity in equilibrium with that of the air. The weight of the remaining substance in each flask was determined by weighing the dishes. The material was then ground and the residues in the four parallel flasks in each series were carefully mixed. After determination of the water content the material in each series was analysed for lignin and cellulose.

Methods of chemical analysis. *Lignin* was determined gravimetrically as lignin-thioglycolic acid by HOLMBERG's method (1930, 1934) as modified by BENGTSSON (1936). The method is based on the fact that in the presence of a strong catalytic acid lignin combines with thioglycolic acid, forming lignin-thioglycolic acid, which is slightly soluble in acids but readily soluble in alkali.

The samples were digested on the water bath with thioglycolic acid dissolved in 2 *N* hydrochloric acid. After the acid solution had been filtered off, dilute potassium hydroxide was added to the undissolved residues, the lignin-thioglycolic acid entering into solution. The alkaline solution was filtered from the residues mainly consisting of cellulose, and the lignin-thioglycolic acid was thereupon precipitated by acidification with 5 *N* hydrochloric acid. The precipitates were taken up in porcelain filter crucibles, and the quantities of ash-free substance were determined by routine methods.

Cellulose was determined by the gravimetric method of CHARPENTIER (1920) modified by BARTHEL and BENGTSSON (1923) as well as by BENGTSSON (1925). The cellulose was extracted from the material with cuprammonium hydroxide (Schweizer's reagent). From a part of the solution the cellulose was thereupon precipitated by addition of 80 % alcohol. For determinations of the cellulose use was made of the undissolved residues of the samples out of which the lignin had been extracted by digestion with thioglycolic acid + hydrochloric acid followed by the treatment with dilute potassium hydroxide.

A detailed description of the methods applied is to be found in LINDEBERG (1944, pp. 18—31).

If the lignin determinations are carried out according to the above-mentioned method on varying quantities of one and the same material, it will be found that the relative yield of lignin-thioglycolic acid increases with the amount of sample. There is however a linear correlation between the quantity of sample and the quantity of lignin-thioglycolic acid found. The reason that the relative yield of lignin-thioglycolic acid increases with the quantity of the sample must therefore be that a constant amount of lignin is lost at every determination. This loss corresponds to the solubility of the lignin-thioglycolic acid in the acid solutions used in the analysis, in which solutions the bulk of the complex mentioned is present as a precipitate.

The quantity in question must be determined for each material, and the amounts of lignin-thioglycolic acid found must then be corrected by addition of the same quantity. This determination is carried out so that the lignin content is ascertained for a series of increasing amounts of substance. A diagram is thereupon plotted in which the dry matter of the samples forms the abscissa and the quantities of lignin-thioglycolic acid found form the ordinates. A series of points are obtained to which is fitted a straight line in such a manner that the sum of squares of deviations in % lignin-thioglycolic acid in the series becomes minimum. This line cuts the vertical axis of the diagram on the negative side. The distance from the point of intersection to origin, rounded to the nearest whole value, is used for the correction mentioned.

In the case of the beech leaves used in the first experimental series the lignin content (as lignin-thioglycolic acid) was determined as 24.6 % (correction + 5 mg) and the cellulose content as 21.7 %. In the second experimental series the values

Table 1.

Lignin and cellulose content of the beech leaves before decomposition.

Dry matter mg	Lignin-thioglycolic acid			Cellulose	
	Found mg	Corrected value mg	%	Found mg	%
a) Material used in the first series.					
258.4	62.7	67.7	26.2	51.2	19.8
275.9	57.6	62.6	22.7	68.2	24.7
286.3	65.4	70.4	24.6	59.4	20.8
383.2	92.9	97.9	25.5	81.0	21.1
451.6	102.6	107.6	23.8	—	—
535.4	116.7	121.7	22.7	118.6	22.2
626.3	155.1	160.1	25.6	137.0	21.9
845.5	210.9	215.9	25.5	188.0	22.2
979.5	234.8	239.8	24.5	200.6	20.5
			24.6		21.7
b) Material used in the second series.					
208.8	44.1	50.1	24.0	—	—
318.9	66.9	72 9	22.9	64.8	20.3
471.8	102.3	108.3	23.0	96.0	20.4
665.8	155.3	161.3	24.2	130.6	19.6
702.7	159.8	165.8	23.6	132.4	18.8
811.8	185.4	191.4	23.6	155.4	19.1
			23.6		19.7

obtained were 23.6 % of lignin (correction + 6 mg) and 19.7 % of cellulose (Table 1). The *Glyceria* straw employed in the first experimental series as a substrate for certain fungi was the same as the writer had used earlier for similar experiments and for which the lignin content (as lignin-thioglycolic acid) had been determined as 13.9 % and the cellulose content as 26.1 % (see LINDEBERG 1944, p. 23).

Two parallel specimens of each of the samples decomposed by the fungi were analysed for lignin and cellulose. With the aid of the values for the lignin and cellulose contents of the residual dry matter the decomposition of lignin and cellulose by the different fungi was then calculated (see Tables 3 and 6).

Of the residual dry matter in the different series, a certain part consisted of mycelium. As it is impossible to separate the mycelium quantitatively from the litter, no correction can be made for this source of error. As BENGTSSON (1938) and the writer (LINDEBERG 1944, pp. 30—31) have shown, the mycelium of fungi contains certain substances (proteins?) which, like lignin, form slightly soluble compounds with thioglycolic acid. The figures showing the lignin contents in Tables 3 and 6 are therefore somewhat too high. However, much seems to indicate that the resultant error in the determinations is rather small (LINDEBERG *l. c.*). Cellulose is practically speaking absent in the mycelium, and the determinations of this substance are therefore not influenced by the presence of the mycelium.

Results.

The investigations comprised two experimental series. In the first series sterilized beech leaves and *Glyceria* straw respectively were inoculated with pure cultures of 36 newly isolated species as well as 7 species belonging to the collection of pure cultures of fungi of the Institute of Physiological Botany of the University of Uppsala, which were courteously placed at the author's disposition by Professor E. MELIN.

After some time it was found that the growth of certain species that had been inoculated on beech leaves was completely or almost completely inhibited. That certain fungi are inhibited in growth by substances that occur in dead leaves of various trees, has been demonstrated by MELIN (1946). MELIN (*l. c.*) also found that the autoclaving of water extracts of certain leaves involves an increased inhibitory effect on the extract. It must be considered as probable that such inhibitory substances have not merely caused the complete inhibition of growth in certain of the species inoculated on beech leaves but that they have also had a depressive effect on the rate of growth of a number of the species with which the experiments could be conducted. In these, the growth-inhibiting substances formed at the autoclaving as well as those naturally occurring have no doubt had an influence of varying degree on the different species. The numerical data obtained in these experimental series for the power of the fungi to decompose sterilized beech leaves must not therefore be immediately regarded as expressing the capability of the different species to attack corresponding material in nature.

The experiments could be carried out with 32 species according to the above-described method. Those fungi which showed good growth on beech leaves all caused a more or less intense decolorization of the leaves. At the same time there was a gradual decrease in the volume of the leaf material.

The results of the first series of experiments are reproduced in Tables 2—4. In this series the experimental period covered

Table 2.

Loss of dry weight in litter caused by the action of the fungi.
First experimental series.

Dry matter per flask before decay	Test-organism	Experimental period in days	Dry matter per flask at end of experiment					Dry-matter decomposed %
			Primary values g				Mean g	
Beech leaves 4.51 g	*Clavaria dendroidea* (Fr. O. K. Fr.	171	3.40	3.34	3.40	3.36	3.38	25.2
	» *gracilis* Fr.	160	3.75	4.13	3.72	4.19	3.95	12.5
	» *ligula* Fr.	171	3.75	—	—	—	3.75	16.9
	Clitocybe Alexandri Fr.	170	3.86	3.84	3.81	3.97	3.87	14.2
	Collybia ambusta Fr.	160	3.80	3.91	3.86	3.85	3.86	14.1
	» *butyracea* Fr.	171	3.12	3.00	2.95	3.83	3.23	28.5
	» *dryophila* Fr.	171	2.76	2.75	2.88	2.95	2.84	37.1
	» *longipes* Fr.	160	3.32	3.37	3.15	—	3.28	27.3
	Cortinellus bulbiger (Fr. Pat.	170	3.53	3.37	3.13	3.21	3.31	26.6
	Flammula carbonaria Fr.	170	3.48	3.42	3.25	3.42	3.39	24.8
	» *penetrans* Fr.	170	3.10	2.79	2.69	2.70	2.82	37.5
	Hypholoma capnoides Fr.	165	2.52	2.57	2.69	2.55	2.58	42.7
	» *fasciculare* Fr.	160	3.17	3.28	3.21	3.11	3.20	29.0
	» *sublateritium* Fr.	165	3.06	3.00	3.08	3.07	3.05	32.3
	Lepiota amianthina Fr.	170	3.17	2.88	3.20	3.16	3.10	31.2
	» *procera* Fr.	170	3.46	3.45	3.52	3.58	3.50	22.3
	Mycena epipterygia Fr.	170	2.89	2.63	2.80	2.68	2.75	39.0
	» *galopoda* Fr.	170	2.79	2.85	2.83	2.75	2.81	37.8
	» *inclinata* Fr.	165	2.48	2.40	2.32	2.40	2.40	46.8
	» *polygramma* Fr.	171	2.30	2.20	2.20	2.30	2.25	50.1
	» *rosella* Fr.	165	3.71	3.68	3.63	4.04	3.77	16.5
	» *viscosa* (Secr. Maire	171	3.46	3.41	3.15	3.32	3.41	24.4
	» *vulgaris* Fr.	171	3.11	2.98	3.26	3.09	3.11	31.0
	Panus stipticus Fr.	160	2.77	2.83	2.93	2.71	2.81	37.7
	Pholiota mutabilis Fr.	160	2.59	2.26	2.66	—	2.50	44.5
	» *squarrosa* Fr.	171	3.05	2.94	2.89	3.01	2.97	34.1
Glyceria straw 4.60 g	*Clitocybe Alexandri* Fr.	167	3.11	3.10	3.11	2.98	3.08	33.2
	» *clavipes* Fr.	167	3.46	3.82	3.10	3.25	3.41	25.9
	» *geotropa* Fr.	167	2.47	2.40	2.34	2.60	2.45	46.7
	» *infundibuliformis* Fr.	167	2.36	2.11	2.25	2.43	2.29	50.3
	» *nebularis* Fr.	167	2.99	3.07	3.06	3.15	3.07	33.3
	» *odora* Fr.	167	3.27	3.10	—	—	3.19	30.8
	Tricholoma gambosum Fr.	165	3.94	3.80	3.77	3.92	3.86	16.1

160—171 days. From Table 2 it is evident that there are great differences between the different fungi in respect of their total attack on the vegetable material. The species that attacked the litter most intensely caused a diminution in dry matter of up to 50 %. The following species broke down more than 30 % of the dry matter:

Table 3.

Decomposition of lignin and cellulose in litter by the action of the fungi.

First experimental series.

Litter	Test-organism	Content of lignin of the decomposed litter %	Lignin decomposed %	Content of cellulose of the decomposed litter %	Cellulose decomposed %
Beech leaves	*Clavaria dendroidea*	16.0	51.2	19.3	33.1
	» *gracilis*	17.1	39.0	24.5	0.8
	» *ligula*	20.1	32.3	23.0	11.5
	Clitocybe Alexandri	19.4	32.3	23.6	6.4
	Collybia ambusta	22.0	23.2	16.6	34.4
	» *butyracea*	8.1	76.5	25.5	15.6
	» *dryophila*	9.4	75.8	10.3	70.1
	» *longipes*	23.3	30.9	12.2	59.1
	Cortinellus bulbiger	15.6	53.3	20.6	30.0
	Flammula carbonaria	20.9	35.9	12.7	56.0
	» *penetrans*	20.3	48.3	25.9	25.3
	Hypholoma capnoides	14.3	66.6	12.7	66.5
	» *fasciculare*	19.7	43.1	12.5	59.1
	» *sublateritium*	18.0	50.3	14.9	53.4
	Lepiota amianthina	16.7	53.3	17.9	43.2
	» *procera*	14.2	55.1	20.0	28.4
	Mycena epipterygia	17.4	56.9	9.3	73.9
	» *galopoda*	20.5	48.1	11.0	68.5
	» *inclinata*	14.5	68.6	7.1	82.6
	» *polygramma*	14.3	70.9	6.2	85.7
	» *rosella*	23.7	19.4	17.2	33.8
	» *viscosa*	13.4	58.7	15.2	47.1
	» *vulgaris*	15.9	55.3	15.8	49.6
	Panus stipticus	15.4	60.9	11.3	67.5
	Pholiota mutabilis	20.4	53.9	10.4	73.4
	» *squarrosa*	19.6	47.4	16.1	51.1
Glyceria straw	*Clitocybe Alexandri*	6.5	68.7	29.7	24.0
	» *clavipes*	11.1	41.0	16.8	52.3
	» *geotropa*	8.8	66.4	7.8	84.0
	» *infundibuliformis*	6.4	77.0	9.2	82.5
	» *nebularis*	7.1	66.0	25.3	35.4
	» *odora*	7.0	65.2	10.6	71.9
	Tricholoma gambosum	13.5	18.7	23.6	24.2

On beech leaves: *Collybia dryophila, Flammula penetrans, Hypholoma capnoides* and *H. sublateritium, Lepiota amianthina, Mycena epipterygia, M. galopoda, M. inclinata, M. polygramma* and *M. vulgaris, Panus stipticus, Pholiota mutabilis* and *Phol. squarrosa*;

On *Glyceria* straw: *Clitocybe Alexandri, Cl. geotropa, Cl. infundibuliformis, Cl. nebularis* and *Cl. odora.*

Among these fungi some can be characterized as wood-destroying fungi (*Flammula penetrans*, the *Hypholoma* species, *Mycena inclinata*, *Panus stipticus* and the *Pholiota* species). It is obvious ,however, that among the specific litter-decomposing fungi there are several species which decompose dead plant residues just as intensely as do the wood-destroying fungi.

Of greater interest than the total loss of dry matter, however, is the reduction that the lignin and the cellulose undergo through the action of the fungi. It may first be pointed out that the 32 species all proved to be capable of decomposing lignin. Several species attacked this substance very intensely. Thus, of the 26 fungi with which the decomposing experiments on beech leaves could be conducted, 15 decomposed more than 50 % of the lignin. This substance was most intensely broken down by the two species *Collybia butyracea* and *Coll. dryophila* (abt. 75 %). It deserves mention that these species are pronounced soil fungi (litter decomposing fungi). The species grown on *Glyceria* straw also caused an intense decomposition of the lignin. *Clitocybe Alexandri*, *Cl. geotropa*, *Cl. infundibuliformis*, *Cl. nebularis* and *Cl. odora* all broke down 65 % or more of the lignin in this material.

The cellulose was also powerfully attacked by several species. Thirteen species decomposed more than 50 % of this substance in beech leaves and four *Clitocybe* species broke down more than 50 % of the same substance in *Glyceria* straw.

An acceptable expression for the specificity of a fungus for the decomposition of one or the other of the two constituents of the plant cell-wall, lignin and cellulose, is obtained by calculating the quantitative relation between the decomposition of lignin on one hand and the decomposition of cellulose on the other, both expressed in percentages. Table 4 presents the quotients obtained in this way for the different fungi.

It will be seen from the table that the value of the quotient *decomposed lignin/decomposed cellulose* for the majority of the species does not deviate much from the value 1. In other words, species that liberate to a high degree one of the two constituents by mainly decomposing the other are relatively few in number.

Very remarkable results, however, were obtained in the cultivation of *Collybia butyracea* on beech leaves. This species decomposed 77 % of the lignin but only 16 % of the cellulose, which caused the lignin content of the material to fall from 25 to 8 %, while the cellulose content rose from 22 to 26 %.

Clitocybe Alexandri also proved to be a highly specific decomposer of lignin. The fungus grew best on *Glyceria* straw and here brought about a fall of the lignin content from 14

Table 4.

List of the quotients $\frac{\text{lignin decomposed (\%)}}{\text{cellulose decomposed (\%)}}$ for the fungi.

Litter	Test-organism	Quotient	Litter	Test-organism	Quotient
Beech leaves	*Clavaria dendroidea* . .	1.5	Beech leaves	*Mycena galopoda*	0.7
	» *gracilis* . . .	*		» *inclinata*	0.8
	» *ligula*	2.8		» *polygramma* . . .	0.8
	Clitocybe Alexandri . .	5.0		» *rosella*	0.6
	Collybia ambusta . . .	0.7		» *viscosa*	1.2
	» *butyracea* . . .	4.9		» *vulgaris*	1.1
	» *dryophila* . . .	1.1		*Panus stipticus*	0.9
	» *longipes* . . .	0.5		*Pholiota mutabilis* . . .	0.7
	Cortinellus bulbiger . .	1.8		» *squarrosa* . . .	0.9
	Flammula carbonaria .	0.6			
	» *penetrans* . .	1.9	*Glyceria* straw	*Clitocybe Alexandri* . . .	2.9
	Hypholoma capnoides .	1.0		» *clavipes*	0.8
	» *fasciculare* .	0.7		» *geotropa*	0.8
	» *sublateritium*	0.9		» *infundibuliformis*	0.9
	Lepiota amianthina . .	1.2		» *nebularis* . . .	1.9
	» *procera*	1.9		» *odora*	0.9
	Mycena epipterygia . .	0.8		*Tricholoma gambosum* . .	0.8

* No cellulose decomposed.

to 7 %, while the cellulose content rose from 26 to 30 %. On being cultivated on beech leaves the fungus showed the same tendency, but its growth was here considerably weaker.

Interesting results were also obtained in the experimental series with the *Clavaria* species. For instance, *Clavaria dendroidea* on beech leaves decomposed 51 % lignin and 33 % cellulose. *Clav. gracilis* on the same substrate caused a total loss of dry matter of 13 %. Of the lignin, 39 % was decomposed, while no cellulose decomposition at all could be definitely established. The development of *Clav. ligula* on beech leaves was much inhibited and very uneven. Only in one of the four parallel flasks did growth get started. In this flask, however, the mycelium thereafter grew through the whole substrate and broke down 32 % of the lignin but only 12 % of the cellulose. A lignin breakdown that was powerful as compared with the cellulose decomposition was also caused by *Cortinellus bulbiger*, *Flammula penetrans* and *Lepiota procera* on beech leaves as well as by *Clitocybe nebularis* on *Glyceria* straw.

On completion of the experimental series described above, another series was started in which attention was given to the following points of view:

1. The results that the three *Clavaria* species examined are more or less specialized for lignin decomposition should be confirmed.

2. The attack on dead plant tissues by certain white-rot fungi among the wood-destroying fungi, *e. g. Polyporus versicolor* and *Pol. abietinus* (CAMPBELL 1930, 1932), should be compared with the effect of the soil fungi on the same material.

3. In view of the fact that intensely lignin-decomposing fungi might conceivably be used for separating lignin from cellulose by biological methods, certain other economically important kinds of plant residues, *e. g.* wheat straw and sawdust, ought to be tested as substrates for the fungi.

4. A valuable control of the experimental method ought to be obtainable by including in the experiment a fungus which earlier investigations have shown to possess the power of breaking down cellulose but not lignin, *e. g. Merulius lacrymans* (FALCK 1926, p. 658).

In the experiment *Clavaria dendroidea, Clav. gracilis* and *Clav. ligula* as well as *Merulius lacrymans, Polyporus abietinus* and *Pol. versicolor* were inoculated on beech leaves. On sterilized wheat straw and sterilized spruce sawdust, firstly, the intensely lignin-decomposing soil fungi *Collybia butyracea, Clitocybe nebularis* and *Marasmius peronatus*, secondly, *Polyporus abietinus* and *Pol. versicolor* were inoculated.

As in the first experimental series *Clavaria dendroidea* showed a more regular growth and a more intense total decomposition of dry matter than the other two *Clavaria* species. On the other hand, the development of *Clavaria ligula* was especially irregular. Only in one of the four flasks did this fungus grow to such an extent that a loss of dry matter could be established. As in the preceding experiment, the contents of this flask were analysed separately without first being mixed with the contents of the other flasks.

Merulius lacrymans developed rather slowly on the sterilized beech leaves, whereas the two *Polyporus* species showed an extremely powerful growth. The last-mentioned fungi both caused a loss of dry matter amounting to more than 50 %.

On wheat straw and spruce sawdust the development of the litter-inhabiting fungi *Clitocybe nebularis, Collybia butyracea* and *Marasmius peronatus* was almost completely inhibited, and no breakdown of the material could be established. On the other hand, the two *Polyporus* species exhibited good development on these substrates as well. Thus *Pol. abietinus* on wheat straw decomposed 22 % and *Pol. versicolor* on the same substrate not less than 50 % of the dry matter. The spruce sawdust was considerably more resistant to attacks of the

Table 5.

Loss of dry weight in litter caused by the action of certain fungi.
Second experimental series.

Dry matter per flask before decay	Test-organism	Experimental period in days	Dry matter per flask at end of experiment					Dry matter decomposed %
			Primary values g				Mean g	
Beech leaves 5.79 g	*Clavaria dendroidea* .	248	4.08	3.52	4.02	4.32	3.99	31.2
	» *gracilis* . . .	209	4.90	4.58	4.72	4.71	4.73	18.2
	» *ligula* . . .	209	4.43	—	—	—	4.43	23.5
	Merulius lacrymans .	187	4.96	4.95	4.86	4.83	4.90	15.4
	Polyporus abietinus. .	205	2.72	2.75	2.49	2.93	2.72	53.0
	» *versicolor* .	183	2.67	2.51	2.71	2.67	2.64	54.4
Wheat straw 5.92 g	*Polyporus abietinus*. .	205	4.45	4.04	5.40	4.61	4.63	21.9
	» *versicolor* .	183	2.92	2.95	2.95	2.94	2.94	50.3
Spruce saw-dust 5.94 g	*Polyporus abietinus*. .	205	5.37	5.37	5.30	5.37	5.35	9.9
	» *versicolor* .	183	5.65	5.69	5.64	5.57	5.64	5.1

fungi. *Pol. abietinus* completely decomposed 10 % and *Pol. versicolor* 5 % of this material. The question whether the inhibition of the litter-inhabiting fungi on wheat straw and sawdust was due to the occurrence in the natural state in these materials of substances which inhibit growth of such fungi or to the formation of inhibitory substances at the autoclaving (*cf.* MELIN 1946) must be left open for the present.

In view of the fact that the soil fungi did not develop on wheat straw and sawdust and no comparison could therefore be made between these fungi and the wood-destroying fungi no lignin or cellulose determinations were carried out on the two materials mentioned. The results of the lignin and cellulose determinations in the experiments in which beech leaves were used as substrate are given in Table 6.

In this experimental series, also, the three *Clavaria* species decomposed considerable quantities of lignin. For instance, *Clav. dendroidea* broke down 62 % of the lignin in 248 days, *Clav. gracilis* 53 % in 209 days and *Clav. ligula* 46 % in the same time. *Clavaria dendroidea* decomposed only 21 % of the cellulose and *Clav. ligula* 24 %. The results of the experiments thus confirm that these two species intensely decompose the

Table 6.

Decomposition of lignin and cellulose in beech leaves by the action of certain fungi.
Second experimental series.

Test-organism	Content of lignin of the decomposed litter %	Lignin decomposed %	Content of cellulose of the decomposed litter %	Cellulose decomposed %
Clavaria dendroidea .	12.9	62.3	22.6	21.1
» *gracilis* . . .	13.6	53.0	25.4	0
» *ligula* . . .	16.8	46.0	19.7	23.7
Merulius lacrymans .	28.8	0	19.1	18.0
Polyporus abietinus .	16.2	67.8	7.3	82.7
» *versicolor* .	13.6	73.7	8.5	80.8

lignin in dead plant tissues while they cause a considerably weaker breakdown of cellulose. The quotient *decomposed lignin* (%)/ *decomposed cellulose* (%) was in this experimental series 3.0 for *Clavaria dendroidea* and 1.9 for *Clav. ligula*. In the preceding experimental series the values of the quotient for the same fungi were 1.5 and 2.8 respectively. It is thus clear that the values determinable by analysis for the quotient *decomposed lignin* / *decomposed cellulose* must not be interpreted as constants, a fact that the author has in fact already stressed (LINDEBERG 1944, p. 34). Probably the relation between lignin and cellulose decomposition in pure culture experiments is dependent amongst other conditions on the nature of the substrate (LINDEBERG *l. c.*) and on the length of the experimental period (*cf.* FALCK 1926, CAMPBELL 1930).

In this series as well no decomposition of cellulose by *Clavaria gracilis* could be established. At the end of the experiment the cellulose content of the residual material amounted to 25.4 %, which means that the total quantity of cellulose in the residual dry matter (totalling 18.91 g) amounted to 4.80 g as against 4.56 g in the initial material. The difference of 5 % probably lies within the limits of error for the experiment. However, it may also be thought that the separation of lignin from cellulose by the thioglycolic acid method is not absolutely complete but that such a fungus as *Clavaria gracilis* liberates further cellulose so that after the action of the fungus on the material this substance is more completely dissolved out by Schweizer's reagent. With this possibility in view the results of these investigations cannot with full certainty be regarded as showing that the fungus does not decompose cellulose. It ought to be possible to decide the question by conducting cultivation experiments with the fungus, in which pure cellulose is added as the only source of carbon. Under all circumstances, among the soil fungi hitherto investigated this species is no doubt most specialized for lignin decomposition.

In the case of *Merulius lacrymans* no lignin decomposition could be demonstrated, and nor was this expected. The lignin content of the residual dry matter represented a total lignin amount which exceeded that of the initial material by 3 %. The agreement was thus good, and the experiment must be considered to afford good evidence of the reliability of the experimental method.

The two *Polyporus* species attacked both lignin and cellulose in beech leaves extremely intensely. Of the lignin about 70 % was decomposed and of the cellulose about 80 %. No enrichment of cellulose was brought about by these species.

Conclusions.

From the results of the present investigation and from the author's earlier published studies respecting Swedish *Marasmius* species (LINDEBERG 1944) it may be inferred that the power of decomposing lignin and cellulose is a very widely occurring property among the soil-inhabiting Hymenomycetes growing on litter. As a matter of fact, species incapable of attacking the lignin but capable of decomposing the cellulose seem to be very few in this group of fungi. Among the genera that include a great number of species capable of decomposing lignin and cellulose special mention may be made of *Marasmius* (LINDEBERG *l. c.*), *Collybia*, *Mycena* and *Clitocybe*. The majority of the species hitherto examined seem to attack lignin and cellulose to about an equal extent, *i. e.* no substantial enrichment in the material of either of these two substances takes place. A few species, however, attack lignin especially strongly but cellulose only to a slight extent. As examples of such species mention may be made of *Collybia butyracea*, *Clitocybe Alexandri*, *Clavaria dendroidea* and *Clav. ligula* as well as the previously examined *Clitocybe nebularis* (FALCK 1930), *Marasmius peronatus*, *M. putillus* and *M. androsaceus* (LINDEBERG 1944). Among the fungi hitherto examined *Clavaria gracilis* occupies a distinct position in that this species attacked the lignin of beech leaves rather intensely while no cellulose decomposition could be established. However, the question whether this species cannot utilize cellulose at all as a source of energy must be left open for the present.

A comparison between the soil fungi and a couple of representatives of the pronounced white-rot fungi among the wood-destroying fungi, *viz. Polyporus abietinus* and *Pol. versicolor*, showed that these last-mentioned fungi attacked the beech-leaves as intensely as the most active among the true soil fungi. However, the two polypori decomposed lignin and cellulose to about the same extent, and therefore no enrichment of cellulose occurred. As a matter of fact, it is evident from the investigations of CAMPBELL (1930, 1931, 1932) and SCHEFFER (1936) that on being grown on wood a large number of wood-destroying fungi attack lignin and cellulose, on the whole, to about an equal extent. Such extreme lignin specialists as occur among the litter-decomposing soil fungi do not seem to be known among the specific wood-destroying fungi at present.

Summary.

1. The capability of certain soil-inhabiting Hymenomycetes to decompose lignin and cellulose in sterilized litter (beech leaves, straw and leaves of *Glyceria maxima*) has been investigated.

2. *Clavaria gracilis* attacked the lignin of beech leaves rather intensely while no cellulose decomposition could be established for this species.

3. The rest of the fungi studied (see Table 2) all decomposed both lignin and cellulose.

4. Some few species attacked lignin rather strongly but cellulose only to a slight extent, *viz. Collybia butyracea, Clitocybe Alexandri, Clavaria dendroidea* and *Clar. ligula*. Most species decomposed lignin and cellulose to about an equal extent.

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